Full papers

Activity and enantioselectivity in the hydrolysis of substituted phenyl esters catalysed by optically active imidazole-containing poly(iminomethylenes)[1,2]

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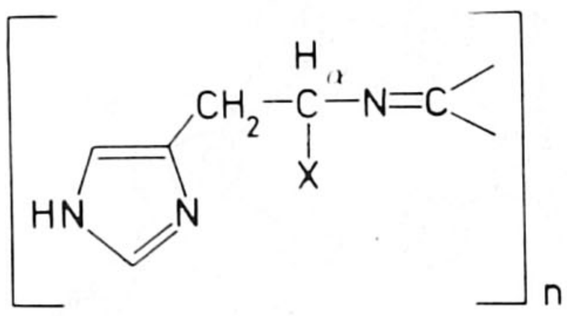
Abstract. The hydrolysis of achiral and chiral esters by optically active poly(iminomethylenes), $[R-N=C<]_n$, which contain imidazole groups in their side chains R, has been studied. The polymers include poly(L- and D-carbylalanyl-L-histidine), **2d** and **2e**, poly(D-carbylalánýl-L-histidinol), **2f**, and poly(L-carbyl-α-methylhistamine), **1c**. The esterolytic catalytic activity of the polymers towards neutral and charged esters increases in the series **2d**, **2e** ≪ **1c** < **2f**. Poly(D-carbylalanyl-L--histidinol) shows enantioselectivity in the hydrolysis of *p*-nitrophenyl esters of D- and L-amino acids, $k_L/k_D = 1.1$.

Introduction

Polymers such as poly(iminomethylenes), $[R-N=C<]_n$, which contain imidazole functions in their side chains R, might resemble, to a slight extent, proteolytic enzymes[3,4]. In earlier papers [4-6] we reported on the synthesis and catalytic activity of this type of polymer. The catalytic role of imidazole in poly(carbylhistidine), **1a**, and in poly-(carbylhistamine), **1b**, was studied in the hydrolysis of charged and uncharged phenyl esters[5]. In particular, the imidazole function of **1a** is more active than the imidazole function of low molecular weight molecules. A cooperative action of the imidazole and carboxylic acid functions in **1a** is held to be responsible for a major part of this activity increase[5]. An imidazole attacks the ester substrate as a nucleophile, while a carboxylic acid group stabilizes the negative charge developing on the carbonyl oxygen. This behaviour slightly resembles that of proteolytic enzymes[7,8].

In view of this similarity we were tempted to investigate, for this type of catalyst, *i.e.* imidazole present in poly-(iminomethylenes), another feature characteristic of enzymes, *viz.* enantioselectivity. Poly(iminomethylenes) have a highly rigid helical configuration[9]. When polymerizing one enantiomer of a chiral isocyanide, polymer molecules are formed with an excess of one screw sense, either right- or left-handed[10,11]. Such rigid chiral structures could induce enantioselective catalysis. This property could not be studied for **1a** and **1b** because the monomer of **1b** is achiral, while the monomer of **1a** racemized during its synthesis. Very recently, we were able to synthesize the optically active poly(iminomethylenes) **1c** and **2d-2f** by a proper choice of monomers and imidazole protecting groups[6,12]. From optical rotation data, as well as from



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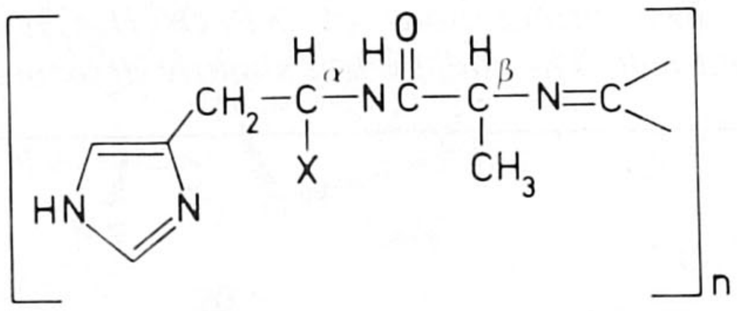

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	X	C^α	C^β
1a	COOH	L,D	
1b	H		
1c	CH_3	L	
2d	COOH	L	L
2e	COOH	L	D
2f	CH_2OH	L	D

Scheme

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[1] Part 16 in the series Poly(iminomethylenes). For Part 15 see ref. 6.
[2] Taken, in part, from the Thesis of *J. M. van der Eijk*, Utrecht, 1980.
[3] For a review on this subject see: *T. Kunitake* and *Y. Okahata*, Adv. Polym. Sci. **20**, 161 (1976).
[4] *J. M. van der Eijk*, *R. J. M. Nolte* and *W. Drenth*, Recl. Trav. Chim. Pays-Bas **97**, 46 (1978).
[5] *J. M. van der Eijk*, *Ch. F. Gusdorf*, *R. J. M. Nolte* and *W. Drenth*, Recl. Trav. Chim. Pays-Bas **98**, 233 (1979).
[6] *J. M. van der Eijk*, *R. J. M. Nolte*, *W. Drenth* and *A. M. F. Hezemans*, Macromolecules, **13**, 1391 (1980).
[7] *J. Drenth*, Recl. Trav. Chim. Pays-Bas **99**, 185 (1980).
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[9] For a review see: *W. Drenth* and *R. J. M. Nolte*, Acc. Chem. Res. **12**, 30 (1979).
[10] *A. J. M. van Beijnen*, *R. J. M. Nolte*, *J. W. Zwikker* and *W. Drenth*, J. Mol. Catal. **4**, 427 (1978).
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circular dichroism spectra, it appeared that these polymers have predominantly one screw sense either right-handed (*P*) or left-handed (*M*). In the present paper we wish to report on the activity and enantioselectivity of **1c** and **2d-2f** in esterolytic catalysis.

Results

The esterolytic activities of the polymeric catalysts towards 2,4-dinitrophenyl acetate, **3**, were measured under conditions of excess imidazole groups at 25.00°C in 29 vol % aqueous ethanol, following the procedure described in ref. 5. All experiments obeyed first order kinetics.
Poly(L-carbylalanyl-L-histidine), **2d**, and poly(D-carbylalanyl-L-histidine), **2e**, are insoluble in the pH range 5–7, since they have their isoelectric points in this region[6,13]. At pH > 7 these polymers showed no significant catalytic activity. The first order rate constants $k_{measd.}$ (with catalyst) and k_{blank} (without catalyst) were equal within experimental error (2%). Further experiments were performed with poly(D-carbylalanyl-L-histidinol), **2f**, and poly(L-carbyl-α-methylhistamine), **1c**[13]. For these polymers the difference between $k_{measd.}$ and k_{blank}, $k_{obsd.} = k_{measd.} - k_{blank}$, was proportional to the molar concentration of imidazole groups, [Cat.]; $k_{obsd.} = k_a$[Cat.]. The second order rate constants, k_a, were measured at various pH values; they are summarized in Table I.

Table I Catalytic rate constants of poly(L-carbyl-α-methylhistamine) (**1c**) *and poly(D-carbylalanyl-L-histidinol)* (**2f**) *in the hydrolysis of 2,4-dinitrophenyl acetate*[a].

pH	$k_a \times 10^2/(dm^3 \cdot mol^{-1} \cdot s^{-1})$	
	1c	**2f**
5.6	36	110
6.3	51	160
7.4	130	310
7.9	190	410
8.5	260	400
9.0	260	420

[a] Solvent 29 vol. % aqueous ethanol. The concentrations of **1c** and **2f** are 3.8×10^{-4} and 4.4×10^{-4} mol imidazole groups/dm^3, respectively; the initial concentration of 2,4-dinitrophenyl acetate is 6.7×10^{-5} mol/dm^3.

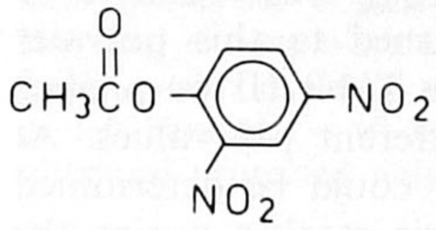
Table II Catalytic rate constants of poly(L-carbyl-α-methylhistamine) (**1c**) *and poly(D-carbylalanyl-L-histidinol)* (**2f**) *in the hydrolysis of a positively charged ester* (**4**) *and a negatively charged ester* (**5**).

Ester	pH	$k_a \times 10^2/(dm^3 \cdot mol^{-1} \cdot s^{-1})$	
		1c	**2f**
4[a]	8.5	2.5	6.3
5[b]	8.5	10.1	18.0
	9.0	13.4	24.8

[a] Solvent water. The concentrations of **1c** and **2f** are 4.7×10^{-4} and 4.5×10^{-4} mol imidazole groups/dm^3, respectively; the initial concentration of **4** is 1×10^{-3} mol/dm^3.
[b] Solvent 29 vol. % aqueous ethanol. The concentrations of **1c** and **2f** are 3.8×10^{-4} and 4.4×10^{-4} mol imidazole groups/dm^3, respectively; the initial concentration of **5** is 1.67×10^{-4} mol/dm^3.

A few similar measurements were performed with the positively charged ester 3-acetoxy-*N*,*N*,*N*-trimethylanilinium iodide, **4**, and the negatively charged ester 4-acetoxy-3-nitrobenzoic acid anion, **5**. The results are given in Table II.

The enantioselectivity of polymers **1c** and **2f** towards chiral esters has been studied in the hydrolysis of 4-nitrophenyl esters of *N*-protected amino acids **6–8**.



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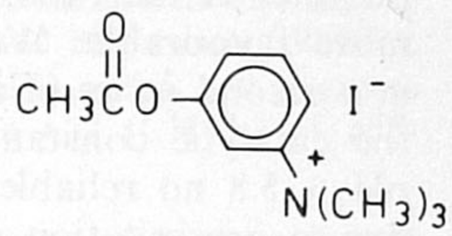

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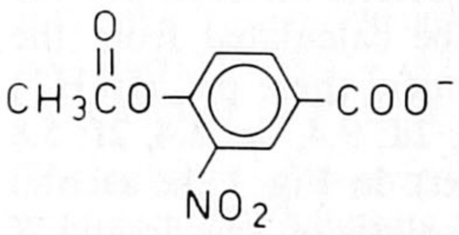

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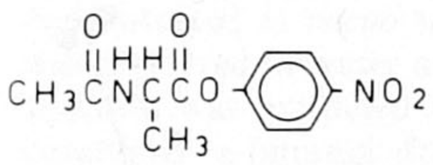
We have chosen these substrates because enantiomers of amino acids are readily available in optically pure form. A disadvantage of esters **6–8** is that in aqueous solution of pH > 7 they rapidly hydrolyze, even in the absence of catalyst. In a first series of experiments we measured the esterolytic activity of polymer **1c** under conditions of excess imidazole groups in water at 25.00°C. Acetate or phosphate buffers were used and the ionic strength was kept constant at 0.02 mol/dm^3. The rates of hydrolysis were determined by following the increase in absorbance of 4-nitrophenol at 320 nm.



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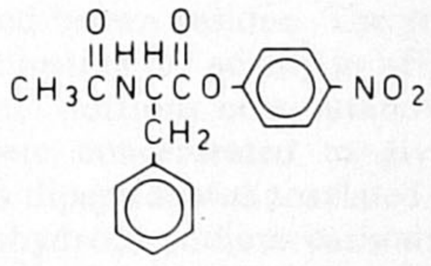

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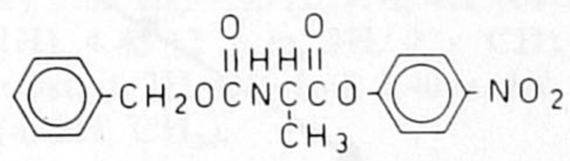
6L, Ac-L-Ala-ONP
6D, Ac-D-Ala-ONP

7L, Ac-L-Phe-ONP
7D, Ac-D-Phe-ONP



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8L, Z-L-Ala-ONP
8D, Z-D-Ala-ONP

[13] According to IUPAC nomenclature rules the polymers are named: **1c**, poly[(*R*)-[2-(4-imidazolyl)-1-methylethyl]carbonimidoyl]; **2d** and **2e**, poly[[(*S* and *R*)-1-[[(*S*)-1-carboxy-2-(4-imidazolyl)ethyl]carbamoyl]ethyl]carbonimidoyl]; **2f**, poly[[(*R*)-1-[[(*S*)-1-(hydroxymethyl)-2-(4-imidazolyl)ethyl]carbamoyl]ethyl]carbonimidoyl].

All experiments followed first-order kinetics. The experiments were performed in duplicate or triplicate. At pH 5.3, 6.1 and 7.2 the difference between the rate constants $k_{measd.}$ and k_{blank} for polymer **1c** appeared to be too small for measuring enantioselectivity accurately. At pH > 7.2 precipitation of the polymeric catalyst occurred, which hampered the measurements at higher pH values. With polymer **2f** the ratio of $k_{measd.}$ to k_{blank} was found to be more favourable. We therefore turned to this polymer in a second series of experiments. In Table III we present the catalytic constants at three different pH values. At pH > 5.8 no reliable rate constant could be determined due to precipitation of the polymeric catalyst during the hydrolysis experiments.

Discussion

Activity. Unprotonated imidazole is the catalytically active species in the hydrolysis of esters. At each pH the fraction of this species, α_{Im}, can be calculated from the pK_a (ImH^+). For the present polymers these pK_a (ImH^+) values are: **1a**: 9.3, **1b**: 5.2, **1c**: 5.5, **2d**: 9.4, **2e**: 8.4, **2f**: 5.8 (see refs. 4, 6 and experimental part). In Fig. 1 the second order rate constants k_a for the hydrolysis of **3** by **1c** and **2f** are plotted against the α_{Im} values calculated at each pH. For comparison a similar plot for polymer **1b**[5] is included.

The three polymers show a linear relationship between k_a and α_{Im} up to $\alpha \approx 0.8$. Above the latter value the curves strongly deviate from linearity in an upwards direction. This behaviour is probably due to a cooperative interaction of neutral imidazole groups along the polymer chain as has been outlined earlier[5] by us. The unprotonated imidazole groups of **1b** and **1c** are almost equally active in the hydrolysis of **3**, $k_a = 0.5\ dm^3 \cdot mol^{-1} \cdot s^{-1}$ ($0 < \alpha_{Im} < 0.8$).

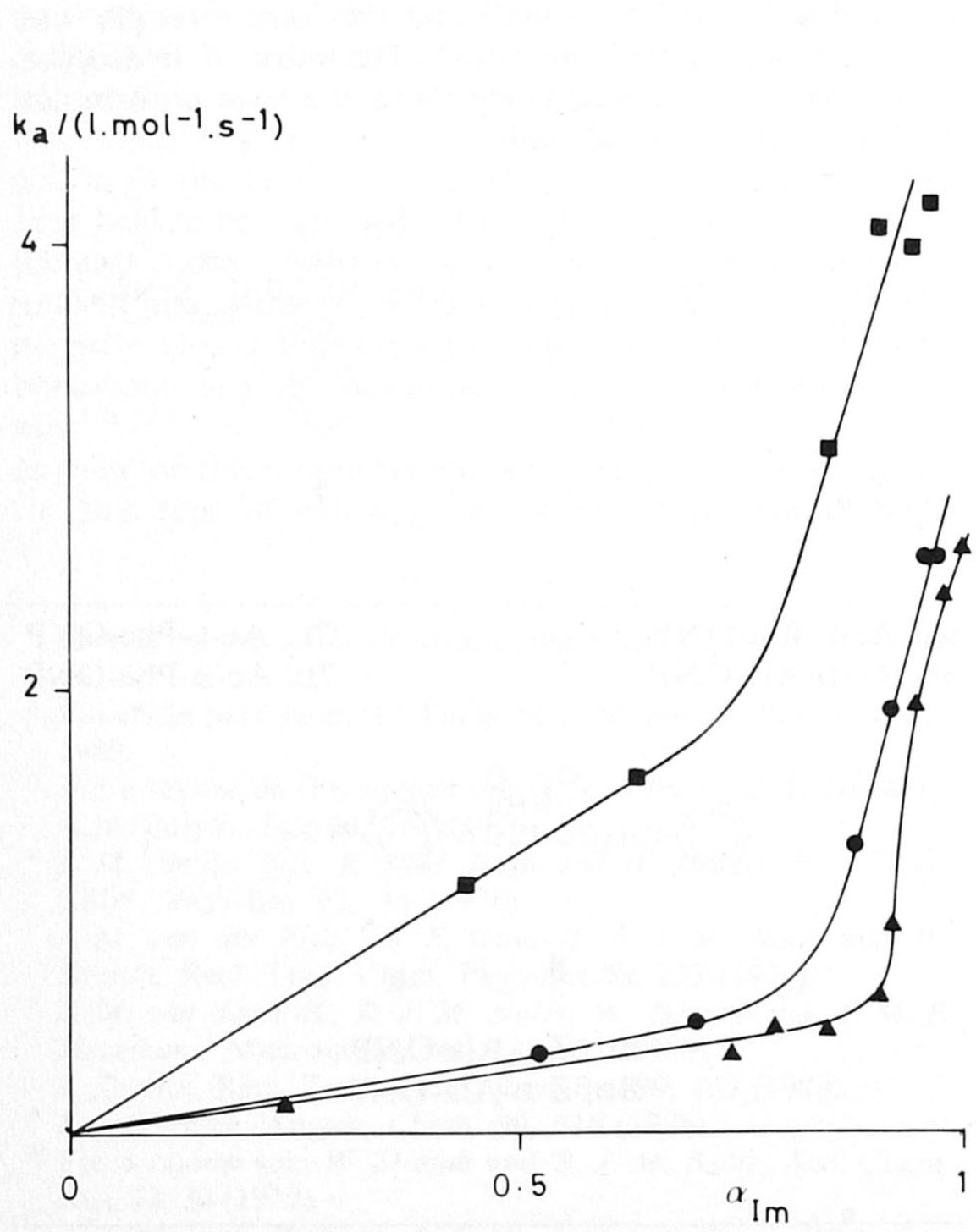

Fig. 1. Catalytic rate constants of poly(carbylhistamine), ▲, *poly(*L*-carbyl-α-methylhistamine),* ●, *and poly(*D*-carbylalanyl-*L*-histidinol),* ■, *in the hydrolysis of 2,4-dinitrophenyl acetate as a function of the fraction of unprotonated imidazole groups,* α_{Im}.

*Table III Catalytic rate constants of poly(*D*-carbylalanyl-*L*-histidinol)* (**2f**) *in the hydrolysis of 4-nitrophenyl esters of optically active amino acids*[a].

Ester	pH 4.7		pH 5.2		pH 5.7	
	$k_a \times 10^2$	k_L/k_D	$k_a \times 10^2$	k_L/k_D	$k_a \times 10^2$	k_L/k_D
Ac-L-Ala-ONP (**6**L)	11.7	1.07	33.5	1.08	52.2	1.11
Ac-D-Ala-ONP (**6**D)	10.9		31.1		46.9	
Ac-L-Phe-ONP (**7**L)	20.2	1.01	52.2	1.08	88.7	1.11
Ac-D-Phe-ONP (**7**D)	20.1		48.4		80.3	
Z-L-Ala-ONP (**8**L)	30.7	1.10	63.3	1.12	104.4	1.07
Z-D-Ala-ONP (**8**D)	28.0		56.1		97.8	

[a] The concentration of polymer **2f** is 1.53×10^{-3} mol imidazole groups/dm^3; the initial concentration of the esters is 5×10^{-5} mol/dm^3. The rate constants k_a are in $dm^3 \cdot mol^{-1} \cdot s^{-1}$; estimeted error 2%.

This activity is very similar to that of the monomeric model compound histamine ($k_a = 0.44\ dm^3 \cdot mol^{-1} \cdot s^{-1}$)[5]. Apparently, the polymeric structure of **1b** and **1c** does not appreciably affect the activity of the imidazole groups in the range $0 < \alpha_{Im} < 0.8$. The unprotonated imidazole group of **2f** is 5 times more active compared to **1b** or **1c**, $k_a = 2.5\ dm^3 \cdot mol^{-1} \cdot s^{-1}$ ($0 < \alpha_{Im} < 0.8$). Possibly, the hydroxymethyl group of **2f** is involved in the catalysis. Such an involvement would be interesting since it is known to occur in proteolytic enzymes[8] but has hardly ever been reported in the literature on polymeric enzyme models[14]. Another explanation might be that the imidazole groups of **2f** are more accessible to substrate molecules than the imidazole groups of **1b** and **1c**. In the latter polymers these groups are close to the polymer main chain. In order to clarify this point it would be necessary to have at our disposal the polymer of carbylhistidinol (**1**, X = CH_2OH). Unfortunately, however, we have not yet been able to synthesize this compound.

In the catalytic hydrolysis of both **5** and **4** polymer **2f** is approximately twice as active as polymers **1b** and **1c**. At the pH's used, the same ratio was found in the hydrolysis of **3** (see Fig. 1 at $\alpha_{Im} \approx 0.95$). From this observation we can conclude that the imidazole groups in the three polymers act similary in the catalytic hydrolysis of charged as well as of uncharged esters.

Polymers **2d** and **2e** are catalytically inactive in the hydrolysis of **3**. This result is striking considering the high activity which was found for the comparable polymer poly(carbylhistidine), **1a**[5]. The difference in catalytic activity between polymers **2d** and **2e** on the one hand, and polymer **1a** on the other hand, might be caused by differences in the conformations of the polymers. Models show that the carboxylic functions of **1a** are situated close to the main chain of the polymer and are forced to interact strongly with each other. The relatively high pK_a (COOH) = 5.8[5] is an indication of this situation. As a result, the imidazole groups are free to point outwards and are easily accessible to substrate molecules. In **2d** and **2e** the carboxylic functions are able to form salt bridges with imidazole groups, making these groups inaccessible to substrate molecules. The relatively low pK_a (COOH) values (**2d**, $pK_a = 2.8$; **2e**, $pK_a = 2.3$) support this hypothesis.

[14] Only one publication deals with this subject. *Overberger* has reported that copolymers of 4-vinylimidazole and vinyl alcohol are 1.2–1.7 times as active as poly(4-vinylimidazole) in the solvolysis of 4-nitrophenyl acetate, *C. G. Overberger, J. C. Salomone* and *S. Yaroslavsky*, J. Am. Chem. Soc. **89**, 6231 (1967).

Enantioselectivity. In Fig. 2 the second order rate constants k_a of polymer **2f** in the hydrolysis of esters **6–8** are plotted against the fraction of unprotonated imidazole groups. In the range studied straight lines were obtained in all cases. For each of the esters, **2f** is slightly more active in its catalysis of the hydrolysis of the L-enantiomer than in that of the D-enantiomer. In view of the consistency in the measurements we can state that polymer **2f** shows enantioselectivity in the hydrolysis of the esters **6–8**. As far as we are aware, this is the first example* of enantioselective catalysis by imidazole anchored to a synthetic polymeric support[15,16]. The enantioselectivity does not noticeably depend on either the structure of the chiral ester or on the pH of the reaction medium. The observed negligible dependence on substrate structure is probably related to a close packing of the side chains in the tightly coiled helical catalyst molecules. As a result of this close packing, substrate molecules are unable to sufficiently penetrate the catalyst for a specific interaction.

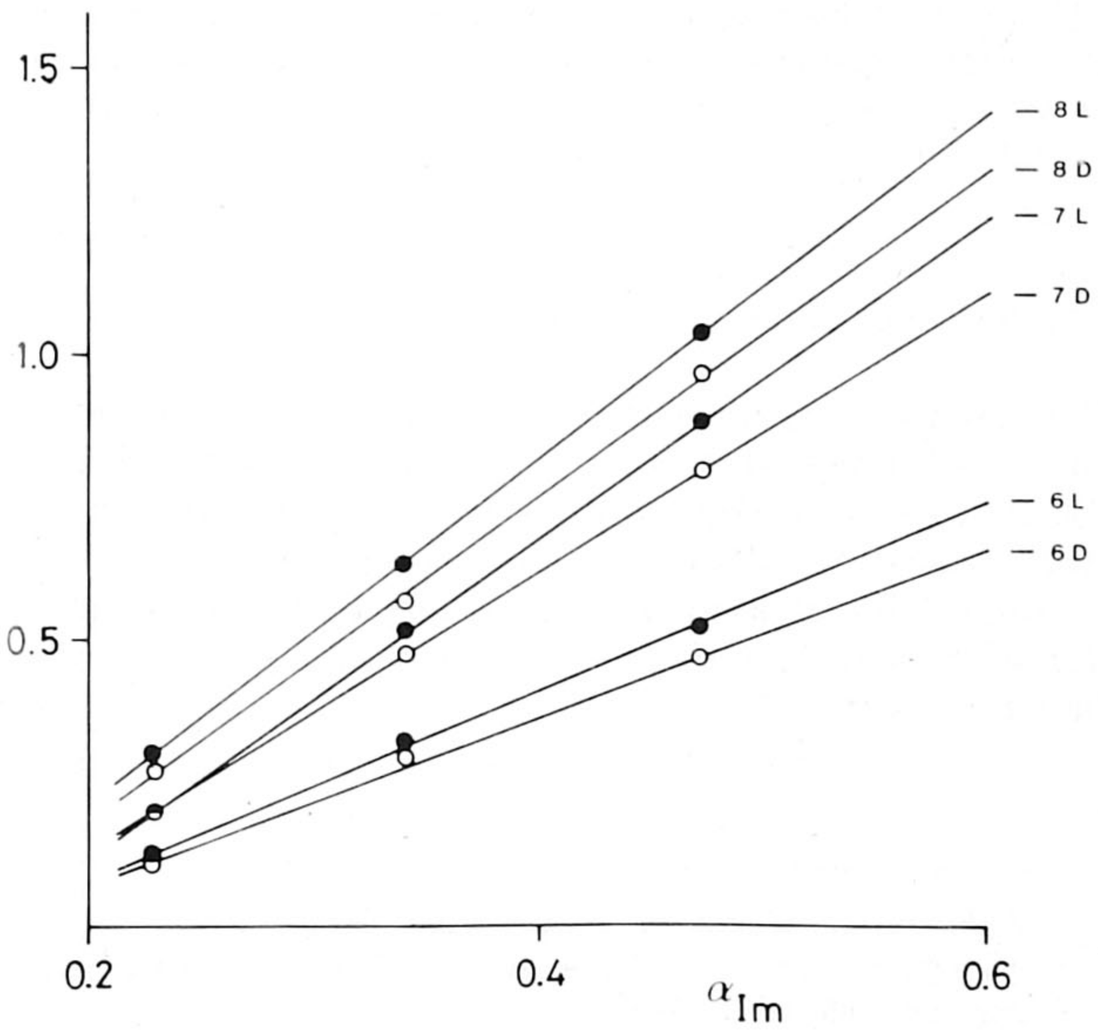

*Fig. 2. Catalytic rate constants of poly(*D*-carbylalanyl-*L*-histidinol) in the hydrolysis of 4-nitrophenyl esters of optically active amino acids* (**6–8**) *as a function of the fraction of unprotonated imidazole groups,* α_{Im}.

In principle, the enantioselectivity of polymer **2f** might be exclusively the result of the enantioselectivity of its chiral side chains. However, up to now enantioselective catalysis by linear dipeptides has not been reported in the literature[17]. At the moment, therefore, we presume that the enantioselectivity of **2f**, at least partly, arises from the helical structure of poly(iminomethylenes).
In this respect it is important to know which helix (*P* or *M*) is in excess in polymer **2f** and to what extent. CD suggests that our sample has an excess of *P*-helices, although a quantitative estimate has not yet proved possible.

Experimental part

Abbreviations used are: s = singlet, d = doublet, q = quartet, m = multiplet, dist. = distorted.

Synthesis

*Poly[(*R*)-carbyl-α-methylhistamine]*, **1c**. The synthesis and physical properties of this polymer will be described elswhere[12]. We used a sample which had: $[\eta]$ = 0.10 dl/g (water, 30.00°); $[\alpha]^{22}_{578}$ −90° (*c* 0.015, water); pK_a (ImH^+) = 5.5 ± 0.1; excess of left-handed (= *M*)-helix according to CD ($CHCl_3$) of N^{Im}-dibenzyl protected polymer[12].

*Poly[*L*-carbylalanyl-*L*-histidine]*, **2d**. This polymer was prepared as described previously[6]. A sample was used which had: $[\eta]$ = 1.72 dl/g (water, 30.00°); $[\alpha]^{22}_{578}$ +350° (*c* 0.05, water); pK_a (ImH^+) = 9.4 ± 0.1, pK_a (COOH) = 2.85 ± 0.1; excess of (*M*)-helix according to optical rotation and probably according to CD[6].

*Poly[*D*-carbylalanyl-*L*-histidine]*, **2e**. This polymer was prepared as described previously[6]. A sample was used which had: $[\eta]$ = 0.15 dl/g (water, 30.00°); $[\alpha]^{22}_{578}$ 0 ± 45° (*c* 0.005, water); pK_a (ImH^+) = 8.4 ± 0.2, pK_a (COOH) = 2.3 ± 0.1; excess of right-handed (= *P*)-helix according to CD[6].

The polymer of D-carbylalanyl-L-histidinol was synthesized according to the following Scheme.

$$\underset{\mathbf{9}}{R'NHCO} \longrightarrow \underset{\mathbf{10}}{R'-N=C} \longrightarrow \underset{\mathbf{2f}}{(R-N=C)_n}$$

$R' = Im(Tos)CH_2CH(CH_2OCOCH_3)NHCOCH(CH_3)$
Im = 4(5)-imidazolyl
$R = ImCH_2CH(CH_2OH)NHCOCH(CH_3)$
Tos = 4-toluenesulfonyl

N-*Formyl-*D*-alanyl-*N*(Im)-tosyl-*O*-acetyl-*L*-histidinol*, **9**. *N*-Formyl-D-alanine[6] [m.p. 131°C, $[\alpha]^{20}_D$ +63° (*c* 2, 1 mol/dm^3 NaOH)] and L-histidinol[18] [dihydrochloride salt: m.p. 197–198°C, $[\alpha]^{22}_D$ −2.9° (*c* 5, water)] were coupled[19] in acetonitrile/*N*,*N*-dimethylformamide (1 : 1 v/v) with dicyclohexyl carbodiimide as coupling agent, following the procedure described previously[6]. The alcohol function of the resulting dipeptide was acetylated with an excess of acetic anhydride in pyridine. After standing overnight at room temperature the reaction mixture was concentrated *in vacuo* to give a red-brown residue. The residue was dissolved in water and after adjusting the acidity to pH 8 the solution was extracted with 50 cm^3 portions of *n*-butanol. The combined *n*-butanol fractions were concentrated to give the acetylated dipeptide as an oil. This dipeptide was tosylated using *p*-toluenesulfonyl chloride and anhydrous sodium carbonate in chloroform/methanol (85 : 15 v/v)[20]. After crystallization from chloroform/ethanol almost pure **9** was obtained. The mother liquid was subjected to column chromatography (eluent $CHCl_3$/acetone, 1 : 1 v/v) to give another crop of pure **9**. Overall yield: 50% (from L-histidinol). White crystals from ethyl acetate: m.p. 126.0–126.2°C; $[\alpha]^{22}_{578}$ +19.2° (*c* 1, $CHCl_3$); IR (KBr): 3300 (NH), 1740 (acetyl), 1600, 1380 and 1180 cm^{-1} (tosyl); ^{1}H NMR ($CDCl_3$): δ 8.10 (s, 1H, CHO), 7.95 and 7.10 (2 × s, 2H, imidazole), 7.85 and 7.35 (2 × d, 4H, tosyl), 7.3 (s, 1H, NH), 7.1 (s, 1H, NH), 4.45 (2 × m, 2H, 2 × CH), 4.05 (dist. d, 2H, CH_2O), 2.75 (dist. d, 2H, CH_2Im), 2.40 (s, 3H, tosyl), 2.00 (s, 3H, acetyl), 1.20 (d, 3H, CH_3).

D-*Carbylalanyl-*N*(Im)-tosyl-*O*-acetyl-*L*-histidinol*, **10**. This compound was obtained by dehydration of **9** with diphosgene[21] and *N*-methylmorpholine following a procedure described pre-

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* Note added in proof: Very recently, *Klotz et al.*[29] have observed enantioselective hydrolysis of amino acid *p*-nitrophenyl esters by optically active poly(ethyleneimines).
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viously[6]. The isocyanide was purified by column chromatography (eluent $CHCl_3$/acetone, 3 : 1 v/v). Yield: 50%. White needles from methanol. M.p. 127.0–127.3°C; $[\alpha]_{578}^{22}$ +3.4° (*c* 1, $CHCl_3$); MS: M^+ 418, M^+–$CH(CH_3)NC$ 364, M^+–CH_2OCOCH_3 345, M^+–tosyl 263; IR (KBr): 3300 (NH), 2148 ± 1 (NC, internal calibration), 1730 (acetyl), 1670 (NHCO), 1250 (acetyl), 1600, 1370 and 1180 cm^{-1} (tosyl); ^1H NMR ($CDCl_3$): δ 8.00 and 7.10 (2 × s, 2H, imidazole), 7.80 and 7.35 (2 × d, 4H, tosyl), 7.8 (d, 1H, NH), 4.45 (m, 1H, $CHCH_2$), 4.15 (q, 1H, $CHCH_3$), 4.05 (d, 2H, CH_2O), 2.80 (d, 2H, CH_2Im), 2.40 (s, 3H, tosyl), 2.00 (s, 3H, acetyl), 1.60 (d, 3H, CH_3).

*Poly(*D*-carbylalanyl-*L*-histidinol)*, **2f**. Isocyanide **10** was polymerized with 0.2 mol % of $NiCl_2 \cdot 6H_2O$ in chloroform/methanol (1 : 1, v/v) as described previously[6]. The polymer obtained was purified by adding a solution of this compound in chloroform to an excess of methanol/water (1 : 1, v/v). Yield 95%; [η] = 0.33 dl/g ($CHCl_3$, 30.00°C), $[\alpha]_{578}^{22}$ −147° (*c* 0.30, $CHCl_3$). Detosylation of the imidazole and hydroxymethyl-protected polymer was performed using acetic anhydride and pyridine (1 : 1, v/v) at room temperature[20]. The impure product was treated with 0.5 mol/dm³ aqueous NaOH for 3 days at room temperature in order to remove the acetyl group. Subsequently, the acidity of the reaction mixture was adjusted to pH 2 with concentrated aqueous HCl, whereafter the solution was subjected to ultrafiltration. Freeze-drying of the resulting, slightly acidified solution afforded **2f** as a dark red solid. Yield 80%; [η] = 0.30 dl/g (water, 30.00°C); $[\alpha]_{578}^{22}$ +70° ± 5° (*c* 0.03, water); Anal.: Calcd. for $C_{10}H_{14}N_4O_2 \cdot (HCl)_{1.12} \cdot (H_2O)_{0.80} \cdot (C_2H_2O)_{0.25}$: C, 43.7; H, 6.0; N, 19.5; O, 17.0; Cl, 13.8. Found: C, 43.7; H, 6.2; N, 19.4; O, 16.9; Cl, 13.8. As revealed by elemental analysis, **2f** still contained 25% of acetyl groups. IR (KBr): 3600–2300 (CH_2OH, protonated imidazole, NH and H_2O), 1650 (NHCO), 1620 cm^{-1} (N=C). A small shoulder on the onset of the amide vibration indicated the presence of the acetylated alcohol function in the polymer; pK_a (ImH^+) = 5.8 ± 0.1; *n*-value (see below) = 2.1 ± 0.05. In the region from 300–400 nm the CD-spectrum of **2f** in water (*c* 1.4 × 10^{-3} mol/dm³) showed a negative couplet superimposed upon a positive Cotton effect; λ (nm), Δε ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$): 310, +0.233; 320, +0.245; 330, +0.170; 340, +0.050; 346, 0.000; 350, −0.015; 360, −0.036; 370, −0.033; 380, −0.015; 390, −0.006; 400, −0.002; 410, 0.000. The CD is due to the *n*-π* transition of the N=C groups in the polymer chain. The negative couplet is indicative of a *P*-helix[11].

Substrates. 2,4-Dinitrophenyl acetate (m.p. 70–71°C), 4-acetoxy-3-nitrobenzoic acid (m.p. 151°C) and 3-acetoxy-*N*,*N*,*N*-trimethylanilinium iodide (m.p. 208°C) were prepared according to literature methods[22-24]. The 4-nitrophenyl esters of *N*-protected amino acids **6–8** were prepared following the procedure of *Ingles* and *Knowles*[25]. These esters had the following physical properties, all of which agree with values reported in the literature: ester, m.p., $[\alpha]_D^{20}$ [1% solution in $CHCl_3$ (**6** and **7**) or ethyl acetate (**8**)]; **6**L, 104–105°C, −68.0°[26]; **6**D, 105–106°C, +68.3°[26]; **7**L, 139–140°C, −18.5°[25]; **7**D, 134–135°C, +17.5°[25]; **8**L, 78–79°C, −33.8°[27]; **8**D, 76–77°C, +32.0°.

Determination of pK_a. Aqueous solutions of the polymers were titrated potentiometrically following a procedure described previously[4]. Evaluation of the titration curves afforded the degrees of dissociation (α) as the function of the pH. Values for pK_a and *n* in the modified Henderson–Hasselbach equation[28]

$$pH = pK_a - n \log [(1 - \alpha)/\alpha]$$

were calculated from plots of $\log [(1 - \alpha)/\alpha]$ *versus* pH.

Kinetics. The kinetic measurements of the hydrolysis of the esters **3**, **4** and **5** were performed essentially as described in ref. 5. In the experiments with the amino acid esters **6–8** the following procedure was used. In aqueous 0.02 mol/dm³ sodium acetate/acetic acid buffers of pH 4.7, 5.2 or 5.7, polymer **2f** was dissolved to a concentration of 1.5 × 10^{-3} mol/dm³. The ester substrates were dissolved in dioxane to a concentration of 1.5 × 10^{-3} mol/dm³. For each measurement 2.9 cm³ of catalyst solution was mixed with 0.1 cm³ of substrate solution in a quartz cell. This cell was subsequently placed in a Cary type 15 spectrophotometer, thermostated at 25.00°C. The absorbance (A_t) of 4-nitrophenol at 320 nm was followed as a function of time. After at least ten half-lives the absorbance corresponding to complete reaction ($A_{t=\infty}$) was measured. Catalysis by buffer only was measured in the same fashion. All experiments were performed in duplicate or triplicate.

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Rearrangement of 5α-hydroperoxy-6-estren-17-one to 6-oxa-B-homo-4,7-estradien-17-one. Preferred migration of the vinyl group

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Abstract. Criegee rearrangement of 5α-hydroperoxy-6-estren-17-one gave 6-oxa-B-homo-4,7-estradien-17-one. This confirms that, in this rearrangement the alkenyl group has a greater migratory aptitude than the alkyl group.

Introduction

Rearrangement of the allylic hydroperoxides, formed by the reaction of singlet oxygen with alkenes, has often been assumed to explain the formation of ketonic side-products[1]. However only in a few cases have the primary rearrangement products been isolated and characterized. *Schenck*

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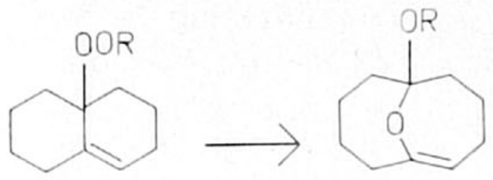
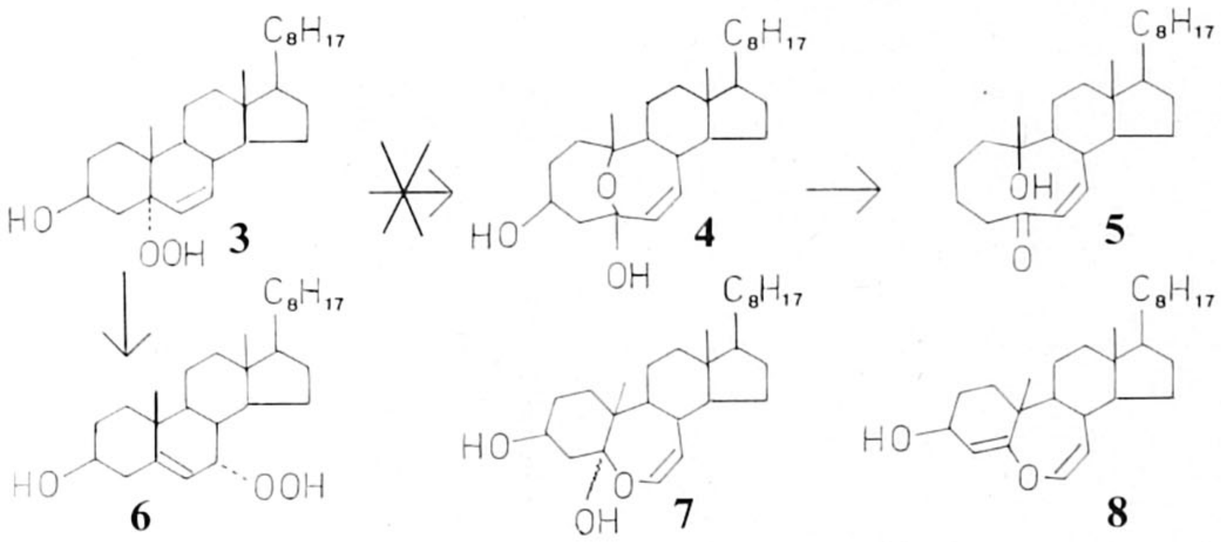
and *Schulte–Elte*[2] have described the rearrangement of the hydroperoxide **1** (R = H) through its 3,5-dinitrobenzoate ester (R = 3,5-dinitrobenzoyl) to the 11-oxabicyclo-[4.4.1]undec-5-ene **2** (R = 3,5-dinitrobenzoyl).



1 **2**

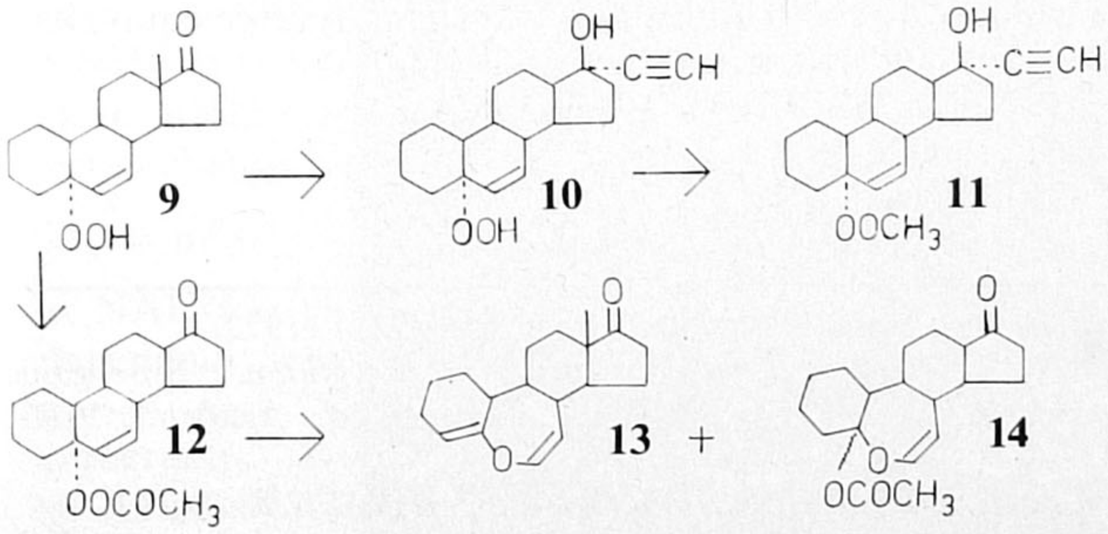
On basis of this result *Schenck, Neumüller* and *Eisfeld*[3] assumed the observed change in optical rotation after storage of a solution of the hydroperoxide **3** in chloroform to be caused by rearrangement to the cyclodecene **5**. The product found however, proved to be the 7α-hydroperoxy--5-cholesten-3-ol **6**. Thus in this solvent 1,3-allylic rearrangement is the preferred reaction and the course of the Criegee rearrangement of these hydroperoxides could not be investigated.



Since the megratory aptitude of an alkenyl group is greater than that of an alkyl group[4] we expect that a Criegee rearrangement would have resulted in the formation of 6-oxa-B-homo derivatives such as **7** or **8** and not in a product like **4**. When we had the opportunity to synthesize 5α-hydroperoxy-6-estren-17-one **9** we decided to study its rearrangement.

Synthesis

Photooxygenation of 5-estren-17-one[5] with hematoporphyrin as sensitizer gave 5α-hydroperoxy-6-estren-17-one **9** as the major product in 53% yield. This hydroperoxide proved fairly stable in alkaline media. It could for example be converted into 5α-hydroperoxy-19-nor-17α-pregn-6-en--20-yn-17-ol **10** with acetylene and potassium *tert*-butoxide in tetrahydrofuran, and then to methyl ether **11** with potassium hydroxide and methyl iodide. Also in weakly acidic media, like acetic acid, no rearrangement was observed. However when the hydroperoxide **9** was converted into the acetate **12** using acetic anhydride and pyridine, this acetate underwent rearrangement to 6 oxa-B--homo-4,7-estradien-17-one **13**. The greater polarization of the O–O bond in the peracetate facilitates the rearrangement[6]. When the rearrangement was carried out at low temperatures (−20°C) 5ξ-acetoxy-6-oxa-B-homo-4,7--estradien-17-one **14** could also be isolated.



That the alkenyl groups had rearranged follows from the NMR spectra of **13** and **14** showing a strong shift in the positions of the vinylic protons (7-H and 7a-H δ 6.23 and 4.50 respectively in **13** and 6.15 and 5.08 in **14**) indicative for the formation of an enol ether[7].

We conclude that during the normal work-up of the hydroperoxides formed by photosensitized oxidation of alkenes, rearrangement is not likely to occur. Under those conditions where Criegee rearrangement is induced the alkenyl group has the strongest tendency to migrate.

Experimental part

Melting points, determined in capillary tubes, are uncorrected. Optical rotations were measured at concentrations of about 1% at 20°C with a Perkin Elmer polarimeter 141. Infrared spectra were recorded on a Perkin Elmer PE-580 grating spectrometer. Proton NMR spectra were recorded with tetramethylsilane as internal standard. Micro analyses were performed by Dr. *W. McMeekin*, Analytical Department, Organon Laboratories, Newhouse, Scotland. Mass spectroscopic measurements were performed on a MS-9 double focussing instrument by Dr. *Bladon*, Strathclyde University, Glasgow.

5α-Hydroperoxy-6-estren-17-one (**9**)

5-Estren-17-one (5.0 g) and hematoporphyrin (0.075 g) were dissolved in pyridine (300 ml). Oxygen was passed through the stirred mixture under irradiation with a 125 W high-pressure mercury lamp. The lamp was installed in a double-walled water-cooled jacket (Duran 50 glass) immersed in the reaction mixture. The temperature was kept around 20°C. The reaction was monitored using thin-layer chromatography. When most of the starting material had been converted, charcoal (Norit) was added and the mixture filtered. The solvent was evaporated under vacuum. The residue (5.0 g) was stirred with methanol and filtered. Evaporation of the filtrate gave 5α-hydroperoxy-6-estren-17-one (3.0 g; 53%) m.p. 165–167°C, sufficiently pure for further synthesis. IR (KBr): 3300 (OH); 1735 (17-oxo); and 1650 (C=C) cm^{-1}. NMR ($CDCl_3$ + 10% CD_3OD): δ 0.84 (s, 18-CH_3); 5.70 and 5.75 (m, 6-H and 7-H). Parent mass 290.1889; calc. for $C_{18}H_{26}O_3$ 290.1882.

5α-Hydroperoxy-19-nor-17α-pregn-6-en-20-yn-17-ol (**10**)

Potassium *tert*-butoxide (3.0 g) was dissolved in dry tetrahydrofuran (30 ml) and at 0°C acetylene was passed through for 2 h. The mixture was cooled to −10°C and a solution of 5α-hydroperoxy-6-estren-17-one (3.0 g) in tetrahydrofuran (30 ml) was added over a period of 30 minutes. At the same temperature acetylene was passed through the mixture for 2 h. The mixture was worked up by pouring into ice–water. The precipitate was collected, washed with water until neutral and crystallized from methanol to give pure 5α-hydroperoxy-19-nor-17α-pregn-6-en-20-yn-17-ol (1.2 g; 33%) with melting point 142–143°, $[\alpha]_D$ −24° (methanol). IR (CH_2Cl_2): 3600 (OH); 3530 (*OOH*); 3300 (C≡*CH*); 1660 (C=C); 1330 (OH); 1042 (C–O); 1015 (C–O) cm^{-1}. NMR ($CDCl_3$): δ 0.86 (s, 18 CH_3); 2.55 (s, C≡CH); 5.78 (s, 6-H + 7-H); 3.45 (s, CH_3OH 1 equivalent).

Analysis: Calc. for $C_{20}H_{28}O_3.CH_3OH$ (348): C 72.38 H 9.26 O 18.37%

Found : C 72.7 H 9.3 O 17.8%

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